Claims 6, 37 and 38 have been canceled. Claims 1, 5, 8, 9, 15, 19, 23, 25-27, 29 and 34 have been amended. Support for the amendments to claim 1 and 8 can be found in Examples 3 and 5 on pages 3 and 4. Support for the amendment to claim 5 can be found in original claim 6. Claims 9 and 23 have been amended to correct typographical errors. Support the amendments to claims 19 and 34 can be found in claims 38 and 37, respectively. Claims 25-27 have been amended as to form.

Claims 39-48 have been added. Support for claims 39-40 and 43 can be found on page 2, lines 2-3; while support for claims 41-42 can be found in original claim 1 and in Examples 1-5 on pages 2-4. Support for claims 44-45 can be found on page 2, lines 18-20 and Examples 3 and 5 on pages 3-4. Support for claim 46 can be found in original claim 1 and on page 2, line 2, while support for claims 37-38 can be found in Examples 1-5 on pages 2-4. As the amendments to the claims are fully supported by the specification as originally filed and add no new matter, entry is believed to be order.

The Examiner has acknowledged Applicants' claims for foreign priority based on an application filed in Sweden on October 21, 1994, however, the Examiner has alleged that Applicants have not filed a certified copy of the Swedish Application.

The current continuing prosecution application based on parent case Serial No. 08/963,288, is a continuation of parent application Serial No. 08/809,256, which claims priority to International Application PCT/SE95/01235, filed October 19,1995. The Notification of Missing Requirements from parent application 08/809,256, dated June 2, 1997, a copy of which is enclosed herein, indicates that the priority document had been received in the parent application by the U.S. Receiving Office. Thus, Applicants submit that no certified copy of the Swedish application is required.

According to claim 1, the invention is directed to an in vitro method of enhancing the transcription of a gene in a DNA construct incorporated into the genome of a eukaryotic host cell, the DNA construct comprising a structural gene for a desired protein or polypeptide and a gene promoter upstream of the structural gene. The method comprises providing upstream of the promoter six copies of an enhancer element comprising the nucleotide sequence TTCTGAGAA, and exposing the DNA construct to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof. The enhancer element is responsive to both lactogenic hormones and somatogenic hormones.

According to claim 5, the invention is directed to an enhancer element which when used in a DNA construct for transfection of a eucaryotic host cell is responsive to hormonal stimuli, said enhancer element comprising the nucleotide sequence TTCTGAGAA, with the proviso that said nucleotide sequence is not the DNA sequence of the SPI-growth hormone responsive element (SPI-GHRE). The enhancer element is responsive to both lactogenic hormones and somatogenic hormones.

According to claim 8, the invention is directed to an expression vector comprising a structural gene encoding a desired protein or polypeptide and a promoter, wherein the vector further comprises six copies of an enhancer element consisting essentially of the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence is not the SPI-growth hormone responsive element (SPI-GHRE).

According to claim 19, the invention is directed to an in vitro method of enhancing the transcription of a gene in a DNA construct comprising a structural gene and a promoter upstream of the structural gene, the method comprising providing upstream of the promoter at least one enhancer element consisting essentially of the nucelotide sequence TTCTGAGAA, and exposing the DNA construct to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof.

According to claim 23, the invention is directed to an enhancer element responsive to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof when the enhancer element is used in a DNA construct for transfection of a eukaryotic host cell. The enhancer element consists essentially of the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence is other than the nucleotide sequence of the SPI-GHRE.

According to claim 27, the invention is directed to an expression vector comprising a structural gene encoding a structural protein, a promoter, and at least one enhancer element consisting essentially of the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence is other than the nucleotide sequence:

GATCTACGCTTCTACTAATCCATGTTCTGAGAAATCATCCAGTCTGCCCATG.

According to claim 34, the invention is directed to an in vitro method of enhancing the transcription of a gene in a DNA construct comprising a structural gene and a promoter upstream of the structural gene. The method comprises providing upstream of the promoter at least one

enhancer element, and exposing the DNA construct to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof. The enhance element consists essentially of the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence is other than the nucleotide sequence:

GATCTACGCTTCTACTAATCCATGTTCTGAGAAATCATCCAGTCTGCCCATG.

According to claim 41, the invention is directed to an in vitro method of enhancing transcription of a structural gene, comprising the steps of:

(a) preparing a plasmid DNA construct comprising a structural gene, a promoter upstream of the structural gene, and at least one enhancer consisting of the sequence TTCTGAGAA upstream of the promoter;

(b) transfecting a cell with the plasmid DNA construct; and

(c) exposing the cell to a hormone selected from the group consisting of growth hormone, prolactin and mixtures thereof.

According to claim 44, the invention is directed to an isolated DNA construct comprising six repeats of an enhancer, wherein the enhancer consists essentially of the sequence TTCTGAGAA.

According to claim 46, the invention is directed to an in vitro method of enhancing the transcription of a gene in a DNA construct incorporated into the genome of a eukaryotic host cell, wherein the DNA construct comprises a structural gene and a gene promoter upstream of the structural gene. The method comprises the steps of:

(a) providing upstream of the promoter at least one copy of the nucleotide sequence TTCTGAGAA, and

(b) exposing the DNA construct to prolactin.

Claims 1, 2, 5-11, 15-17 and 19-38 have been rejected under 35 U.S.C. §112 as not being supported by an enabling specification. The Examiner alleges that the specification, while being enabling for a method of enhancing transcription in vitro using an SPI-growth hormone responsive element and lactogenic stimuli, does not provide enablement for any method of enhancing transcription in vitro, or method of enhancing transcription in vitro or in vivo using any and all enhancer elements comprising the nucleotide sequence TTCTGAGAA and exposing the DNA construct to lactogenic stimuli. The Examiner alleges claims 19-38 are further rejected

on the grounds of the specification does not teach how to use the claimed enhancer element, expression vectors, or DNA constructs for anything other than in vivo applications.

As will be set forth below, Applicants submit that claims 1-2, 5-11, 15-17 and 19-38 are supported by an enabling specification. Accordingly, the rejection is traversed, and reconsideration is respectfully requested.

The Examiner has alleged the specification fails to provide an enabling disclosure for methods of enhancing the transcription in vitro using any enhancer element comprising the nucleotide sequence TTCTGAGAA because one cannot predict whether any and all enhancers comprising the nucleotide sequence would be responsive. Claims 1 and 5, and the claims dependent thereon, are limited to sequences comprising the core element TTCTGAGAA which are responsive to both lactogenic and somatogenic stimuli. Thus, sequences which are not responsive are excluded. One of ordinary skill could easily determine whether sequences are responsive to hormonal stimuli by, for example, preparing constructs expressing protein such as bacterial protein chloramphenicol acetyl transferase or firefly luciferase, incubating cells containing the DNA construct overnight, lysing the cells and measuring enzyme activities, as set forth in Example 2.

Claim 8, 19, 27 and 34 recite enhancer elements consisting essentially of the nucleotide sequence TTCTGAGAA Thus, the claims exclude sequences which do not enhance transcription, for such sequences would materially affect the basic and novel characteristics of the claimed invention. As discussed above, one of ordinary skill could easily determine whether sequences are responsive to hormonal stimuli by, for example, preparing constructs expressing protein such as bacterial protein chloramphenicol acetyl transferase or firefly luciferase, incubating cells containing the DNA construct overnight, lysing the cells and measuring enzyme activities, as set forth in Example 2.

Claims 19-38 have been further rejected under 35 U.S.C. §112, first paragraph based on the Examiner's allegation that the specification does not teach how to use the claim enhancer element, expression vectors, or DNA constructs for anything other than in vivo applications. However, Examples 2, 4 and 5 on pages 3-4 disclose in vitro uses of expression vectors and enhancer elements. For example, Example 2 on page 3 discloses that expression plasmid were prepared containing six repeats of the 50 base pair growth hormone responsive element, while corresponding constructs were prepared using the 9 base pair enhancer element. The variants

expressed in a bacterial protein chloramphenicol acetyl transferase or firefly luciferase. The plasmids, along with plasmid expression vectors encoding hormone receptors or mouse prolactin receptors, were transfected into eukaryotic cells such as the Chinese hamster ovary or buffalo rack liver cells. Exposure of the cells to growth hormone or prolactin shows an increase in chloramphenicol acetyl transferase or luciferase enzyme activity. Thus, the expression vectors and enhancer elements may be used to increase production of transfected enzyme genes, thereby allowing the study of enzyme activities and the isolation of the produced enzyme.

Thus, for the reasons set forth above, Applicants submit that claims 1, 2, 5-11, 15-17 and 19-38 are supported by an enabling specification, whereby the rejection under 35 U.S.C. §112, first paragraph, should be withdrawn.

Claims 9 and 10 have been rejected under 35 U.S.C. §112, first paragraph as containing subject matter not described in the specification. The Examiner notes that the claims are directed toward a "thiamine" kinase promoter while the specification refers to a "thymidine" kinase promoter. The typographical error in claim 9 has been corrected, whereby the rejection has been overcome.

Claims 9, 10 and 23 have been rejected under 35 U.S.C. §112, second paragraph as being indefinite. The Examiner alleges claims 9 and 10 are indefinite in the recitation of a thiamine kinase promoter and claim 23 is indefinite in its recitation of transection, which appears to be a typographical error which should read "transfection". The typographical errors have been corrected, whereby the rejection of claims 9-10 and 23 under 35 U.S.C. §112, second paragraph has been overcome.

Claims 1-2 have been rejected under 35 U.S.C. §102 as being anticipated by Yoon, et al., 1990. The Examiner alleges Yoon, et al. teach transcription of serine protease inhibitor (SPI 2.1) gene is induced by growth hormone, and that Yoon, et al. further teach the isolation and characteristic of the SPI 2.1 gene from rat genomic library. The Examiner alleges Yoon, et al. teach that portions of the 5′ flanking region of the gene were fused to a heterologous promoter and reporter gene, and introduced into hepatocytes thereby generating expression vectors in eukaryotic host cells. The Examiner further alleges Yoon, et al. teach that a -147 to -102 segment of the gene could confirm growth hormone responsiveness when linked in tandem copies in front of a heterologous promoter.

As set forth below, Applicants submit that claims 1-2 are not anticipated by Yoon, et al. Accordingly, the rejection is transversed and reconsideration is respectfully requested.

Yoon, et al. teach that transcription of the SP 2.1 gene is induced by growth hormone, and when portions of the 5′ flanking regions are fused heterologous promoter and reporter genes introduced into hepatocytes, there is a two- to threefold induction of reporter gene activity in the cells grown in the presence of growth hormone. Yoon, et al. also teach that further definition of the essential sequences reveal that a segment from -147 to -102 could confer growth hormone responsiveness, and sets forth the nucleotide sequence of this segment. Applicants find no teaching or suggestion in Yoon, et al. of a method of providing six copies of an enhancer element comprising the nucleotide sequence TTCTGAGAA, particularly wherein the enhancer is responsive to both lactogenic hormones and somatogenic hormones, as set forth in claim 1.

Therefore, for the reasons set forth above, Applicants submit that claim 1, and claim 2 dependent thereon, are not anticipated by Yoon, et al., whereby the rejection should be withdrawn.

Claims 5-11, 15-17 and 23-33 have been rejected under 35 U.S.C. §103 as being unpatentable over Lindquester, et al. (1989). The Examiner alleges Lindquester, et al. disclose a nucleotide sequence of a avian tropomyosin gene which includes a sequence TTCTGAGAA located on one of the introns. The Examiner alleges that since enhancer elements are known to be located on introns, the sequence TTCTGAGAA on an intron will function as an enhancer element, and that the hormone responsiveness of the element is an inherent property of the element. The Examiner further alleges one would have been motivated to construct an expression vector comprising the tropomyosin gene and host cell comprising the expression vector in order to produce the protein in culture.

As will be set forth below, Applicants submit that claims 5-11, 15-17 and 23-33 are not rendered obvious by Lindquester, et al. Accordingly, the rejection is transversed and reconsideration is respectfully requested.

Lindquester, et al. disclose sequence analyses of overlapping fragments of quail genomotic library were used to determine a tropomyosin gene consisting of 13 exons spaced over about 18 kilobase pairs of DNA. An intron of the avain tropomyosin gene contains the sequence TTCTGAGAA. Lindquester, et al. disclosed that one set of exons expressed in skeletal muscle and another set of exons is expressed in smooth muscle.

Applicants find no teaching or suggestion in Lindquester, et al. that the DNA sequence TTCTGAGAA may serve as an enhancer element which is responsive to both lactogenic hormones and somatogenic hormones, as recited in claim 5 and claims dependent thereon. Inherency and obviousness are entirely different concepts and the view that success would have been inherent cannot substitute for a showing of reasonable expectation of success. *In re Rienhart*, 189 USPQ 143 (CCPA 1976).

Further, Applicants find no teaching or suggestion in Lindquester, et al. of an expression vector comprising six copies of an enhancer element consisting essentially of the nucleotide sequence TTCTGAGAA, as recited in claim 8 and claims dependent thereon.

Applicants find no teaching or suggestion in Lindquester, et al. of an enhancer element consisting essentially of the nucleotide sequence TTCTGAGAA which is responsive to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones, and mixtures thereof, when the enhancer is used in a DNA construct for transfection of a eukaryotic host cell, as recited in claim 23 and claims dependent thereon.

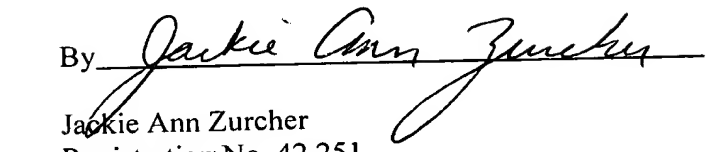
Applicants find no teaching or suggestion in Lindquester, et al. of an expression vector comprising at least one enhancer element consisting essentially of the nucleotide sequence TTCTGAGAA, as set forth in claim 27 and claims dependent thereon, or of an expression vector comprising an enhancer element consisting essentially of a nucleotide sequence TTCTGAGAA, wherein the enhancer element is responsive to hormones selected from the group consisting of prolactin placenta, lactogen and mixtures thereof, as recited in claim 29.

Therefore, for the reasons set forth above, Applicants submit that claims 5-11, 15-17 and 23-33 are not rendered obvious by Lindquester, et al., whereby the rejection should be withdrawn.

For the reasons set forth above, Applicants submit that claims 1-2, 5-11, 13-17 and 19-38 are definite and supported by enabling disclosure, and are neither anticipated by Yoon, et al. nor rendered obvious by Lindquester, et al. The Examiner is therefore requested to withdraw the rejections to claims 1-2, 5-11, 5-17 and 19-38, and to allow the application to pass to issue.

Respectfully submitted,

GUNNAR NORSTEDT ET AL.

By 

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